PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :		(11) International Publication Number:
A61K 31/70, C07H 15/12, 17/00	A1	WO 93/09788
		(43) International Publication Date: 27 May 1993 (27.05.93)

(21) International Application Number: PCT/US92/09202

(22) International Filing Date: 28 October 1992 (28.10.92)

(30) Priority data:
07/792,319 13 November 1991 (13.11.91) US

(71) Applicant: BAYLOR COLLEGE OF MEDICINE [US/US]; One Baylor Plaza, Houston, TX 77030-3498 (US).

(72) Inventor: HOGAN, Michael, Edward ; 103 Golden Shadow Circle, The Woodlands, TX 77381 (US).

(74) Agent: PAUL, Thomas, D.; Fulbright & Jaworski, 1301 McKinney, Suite 5100, Houston, TX 77010-3095 (US).

(81) Designated States: CA, JP, NO, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, SE).

Published
With international search report.

(54) Title: TRIPLEX FORMING OLIGONUCLEOTIDE REAGENTS TARGETED TO THE *NEU* ONCOGENE PROMOTER AND METHOD OF USE

(57) Abstract

A method for inhibiting the proliferation of cells which contain an erb B2/*neu* gene site. The method involves administering a therapeutic dose of an oligonucleotide which is capable of forming a colinear triplex with the promoter region of the erb B2/*neu* gene. The oligonucleotides can inhibit the CAT box, the TATA box or the linker domain between the CAT and TATA box or any combination of the these three. Specific oligonucleotides which will bind and form triplexes in this region are described.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria
AU	Australia
BB	Barbados
BE	Belgium
BF	Burkina Faso
BG	Bulgaria
BJ	Benin
BR	Brazil
CA	Canada
CF	Central African Republic
CG	Congo
CH	Switzerland
CI	Côte d'Ivoire
CM	Cameroon
CS	Czechoslovakia
CZ	Czech Republic
DE	Germany
DK	Denmark
ES	Spain
FI	Finland
FR	France
GA	Gabon
GB	United Kingdom
GN	Guinea
GR	Greece
HU	Hungary
IE	Ireland
IT	Italy
JP	Japan
KP	Democratic People's Republic of Korea
KR	Republic of Korea
KZ	Kazakhstan
LI	Liechtenstein
LK	Sri Lanka
LU	Luxembourg
MC	Monaco
MG	Madagascar
ML	Mali
MN	Mongolia
MR	Mauritania
MW	Malawi
NL	Netherlands
NO	Norway
NZ	New Zealand
PL	Poland
PT	Portugal
RO	Romania
RU	Russian Federation
SD	Sudan
SE	Sweden
SK	Slovak Republic
SN	Senegal
SU	Soviet Union
TD	Chad
TG	Togo
UA	Ukraine
US	United States of America
VN	Viet Nam

TRIPLEX FORMING OLIGONUCLEOTIDE REAGENTS TARGETED TO THE *NEU* ONCOGENE PROMOTER AND METHOD OF USE

FIELD OF INVENTION

The present invention relates generally to a method of inhibiting the growth of cells using triplex forming oligonucleotides. More particularly it relates to the inhibition of cell growth using oligonucleotides which form triplexes to the promoter region of the erb B2/*neu* gene. It also relates to specific triplex forming oligonucleotides.

BACKGROUND OF THE INVENTION

The c-erb B2/*neu* (HER-2) gene is the human homologue of the rat *neu* gene. *Neu* was originally identified in the rat in ethylnitrosourea transformed neuroblastomas. Subsequently, it has been shown that the human homologue is frequently amplified in tumors. When expressed at high levels in NIH3T3 cells, c-erb B2/*neu* is strongly transforming and results in a high incidence of mammary tumors in transgenic mice. The efficiency of tumor induction suggests that overexpression of the gene is a sufficient condition (i.e., the cause) of breast cancers in these transgenic mice. Amplification of c-erb B2/*neu* has been strongly correlated with poor patient prognosis.

The core promoter element of erb B2/*neu* resides within a 300 BP region of the 5′ flanking domain. This region contains promoter-enhancer elements which confer sensitivity to enhanced promoter function in the presence of cell growth factors such as TPA, c-AMP and retinoic acid.

Based upon those data, it can be argued that over expression of erb B2/*neu* may be one mechanism leading to cancer initiation or progression. As such, pharmaceutical agents which selectively inhibit erb B2/*neu* expression may selectively inhibit the tumorigenicity of cells.

It is known that synthetic oligonucleotides can be designed to form triplex helices with DNA. U.S. Applications Serial No. 7/453,532 Hogan & Kessler filed December 22, 1989. This application describes a method for making synthetic oligonucleotides which are targeted to specific sequences in duplex DNA and which form colinear triplexes by binding to the major groove of the DNA duplex. Alternative methods of forming triplex molecules with varying degrees of success have been described.

The present invention provides a series of novel oligonucleotides targeted to the erb B2/*neu* gene and a novel method for treating proliferative growth of cells containing the erb B2/*neu* gene. These oligonucleotides and method can be used to treat cancer which results from abnormalities in the regulation of the erb B2/*neu* gene and in other non-malignant diseases such as psoriasis and scarring.

SUMMARY OF THE INVENTION

An object of the present invention is a method for inhibiting proliferating cells with triplex forming oligonucleotides specific to the promoter region of the erb B2/*neu* gene site.

A further object of the present invention is a method of treating cancer.

An additional object of the present invention is the provision of triplex forming oligonucleotides which are anti-cancer agents.

Thus, in accomplishing the foregoing objects, there is provided in accordance with one aspect of the present invention a method for inhibiting the proliferation of cells containing an erb B2/*neu* gene site comprising the step of administering a therapeutic dose of an

oligonucleotide, said oligonucleotide capable of binding to the major groove of a duplex DNA to form a colinear triplex with the promoter region of the erb B2/*neu* gene.

In specific embodiments of the invention the oligonucleotides can bind in the promoter region to the CAT box, the TATA box or the linking domain connecting the CAT box and TATA box.

Specific oligonucleotides which bind to the CAT box, the TATA box, the linking domain or a combination thereof are described.

In the preferred embodiment the oligonucleotides are capped at the 3′ terminus with a polyamine, a cholesterol or a poly-L-lysine modifier.

DETAILED DESCRIPTION

It is readily apparent to one skilled in the art that various substitutions and modifications may be made to the invention disclosed herein without departing from the scope and spirit of the invention.

The term "oligonucleotide" as used herein is defined as a molecule comprised of two or more deoxyribonucleotides or ribonucleotides, preferably more than ten. Its exact size will depend on many factors including the specificity and binding affinity.

When referring to "bases" herein the term includes both deoxyribonucleic acids and ribonucleic acids. The following abbreviations are used: "A" refers to adenine as well as its deoxyribose derivatives, "T" refers to thymine as well as its deoxyribose derivatives, "G" refers to guanine as well as its deoxyribose derivatives, "C" refers to cytosine as well as its deoxyribose derivatives.

The "major groove" refers to one of the grooves along the outer surface of the DNA helix which is formed because the sugar-phosphate backbone extends further from the axis than the bases. The major groove is important for the binding of regulatory molecules to specific DNA sequences.

The term "triplex forming oligonucleotide" or "TFO" is used herein to refer to the oligonucleotides of the present invention which are capable of forming a triplex by binding to the major groove of a duplex DNA structure.

The term "inhibition" of the growth of proliferating cells is meant to include partial and total growth inhibition and to include decreases in the rate of proliferation or growth of the cells. The inhibitory dose or "therapeutic dose" of the compounds in the present invention may be determined by assessing the effects of the oligonucleotide on cell growth in tissue culture or tissue growth in an animal. The amount of oligonucleotide administered in a therapeutic dose is dependent upon the age, weight, kind of concurrent treatment and nature of the cell growth condition being treated. The amount of oligonucleotide in a therapeutic dose will include a sufficient amount to account for cellular uptake and binding to the promoter site in the erb B2/*neu* gene.

One embodiment of the present invention is a method for inhibiting the proliferation of cells containing an erb B2/*neu* gene site, comprising the step of administering a therapeutic dose of a oligonucleotide. The oligonucleotide is capable of binding to the major groove of a DNA triplex to form a colinear triplex with promoter region of the erb B2/*neu* gene.

One strand of the duplex DNA of the promoter region of the erb B2/*neu* gene is shown in SEQ. ID. NO. 1. This strand is the purine rich strand. The target site on the duplex DNA should have a stretch of DNA which is at least 65% purine or pyrimidine bases. The TFO which is selected to bond to this region should be at least 20 nucleotides long. The TFO is complementary to the identified target sequence. The TFO includes a G when the complementary location in the duplex DNA is a GC base pair and T when the complementary location in the duplex DNA is an AT base pair. The sequence can be either oriented 3′ to 5′ and bind anti-parallel to the at least 65% purine strand of the duplex DNA target

sequence or be oriented 5′ to 3′ and bind parallel to the at least 65% purine strand of the duplex DNA target. Although oligonucleotides which do not comply with these parameters can form triplexes, the deviation will effect the binding affinity and site specificity of the oligonucleotides and consequently the biological potency of the oligonucleotide. These altered oligonucleotides will usually be inferior to the oligonucleotides having the G/GC and a T/AT relationship. One skilled in the art will readily recognize that alterations to the bases, end capping and altered backbone structure will also affect triplex formation.

The erb B2/*neu* gene is a useful target because of its role in cancer and because its core promoter region SEQ. ID. NO. 1 possesses a long polypurine run. This long polypurine run serves as the linkage between the CAT box and the TATA box. In physical terms the polypurine rich sequences form the best target site for TFO's. Therefore, the promoter region of erb B2/*neu* provides an example of direct overlap between sites of transcriptional importance and an excellent, high infinity, selective TFO binding site.

Inhibition of protein binding at the CAT box transcription factor binding site will inhibit transcription initiation by interfering directly with the CAT box protein-RNA polymerase interaction. Further, inhibition of the protein binding at CAT box site can also block the interaction of the CAT protein with TFIId at the TATA box binding site.

Inhibition of protein binding at the TATA box binding domain (TFIId binding site) will inhibit transcription initiation by interfering directly with the TFIId-RNA polymerase interaction. Further, inhibition of the TATA box site can also inhibit direct interaction with RNA polymerase.

Within this long stretch of polypurine there can also be TFO binding to the linker domain which connects the CAT box and TATA box sites. The spacing between the CAT box and TATA box is important for

the actual functioning of this promoter site. Binding to the linker domain with a TFO of sufficient length will disrupt the CAT protein-TFIId interaction by altering the shape and stiffness of the linker domain.

Thus the erb B2/*neu* gene can be regulated by either TFO binding to the CAT box, TATA box or linker domain. Additionally, TFO's which overlap these domains can be synthesized. Thus a TFO can bind to a combination of sites within this promoter region to effectuate regulation and inhibition of the erb B2/*neu* gene.

Examples of sequences which can intervene with the proliferation of cells containing the erb B2/*neu* gene site are the anti-parallel sequences shown in SEQ. ID. NO. 2, SEQ. ID. NO. 3 and SEQ. ID. NO. 4 and the parallel sequences shown in SEQ. ID. NO. 5, SEQ. ID. NO. 6 and SEQ. ID. NO. 7. SEQ. ID. NOS. 2 and 5 are 54 base pair length oligonucleotides which are capable of CAT box, TATA box and linker mediated inhibition of the promoter region. SEQ. ID. NOS. 3 and 6 are 41 base pair length oligonucleotides which are capable of TATA box and linker mediated inhibition. SEQ. ID. NOS. 4 and 7 are 28 base pair length oligonucleotides which are capable of CAT box and linker mediated inhibition.

The inhibition of the promoter region can be further enhanced by using 3′ or 5′ modifications, substitution with inosine, xanthine or other nucleotides or internal base modifications or by altering the backbone. The sequences shown in SEQ. ID. Nos. 2-7, however, show excellent binding properties and selectivity and are thus excellent for inhibiting the expression of the erb B2/*neu* gene.

In the preferred embodiment, SEQ. ID. Nos. 2-7 have a 3′ terminus modification. The capping of the 3′ terminus with the modifier reduces the oligonucleotide's sensitivity to 3′ exonucleases, thus increasing its biological half-life. The amine modifier can be selected from the group

consisting of polyamine, poly-L-lysine and cholesterol. In one embodiment propylamine is the 3′ cap.

In a preferred embodiment these oligonucleotides are useful in the treatment of cancers. Examples of some cancers which can respond to treatment are neuroblastoma, glioblastoma, breast adenocarcinoma and other tumors related to erb B2/*neu* gene expression. Thus, the TFOs which selectively inhibit the erb B2/*neu* promoter region can be used to slow the growth of transformed cells and in certain instances reverse the transformation process.

Although the erb B2/*neu* gene function in normal cells in not completely understood, its protein product is a kinase. This protein kinase is very similar to the epidermal growth factor receptor and probably serves a related function. Thus, the anti-*neu* TFOs can also be useful as inhibitors of untransformed cell growth. Examples of cell growth which can be regulated include scarring and lesions such as psoriasis.

The following example is offered by way of illustration and is not intended to limit the invention in any manner.

EXAMPLE 1

Band Shift Analysis

The disassociation constants (Kdiss) for SEQ. ID. Nos. 2-4 were measured by band shift analysis. Each TFO was added to the promoter binding site (SEQ. ID. NO. 1) as a duplex with its Watson/Crick complement. The conditions were 10 mM Tris/HCl at pH 7.8 and 10μM $Mg\ Cl_2$.

The results are shown in Table 1.

Table 1

Band Shift Analysis

TFO	Kdiss
SEQ. ID. NO. 2	3×10^{-8} M
SEQ. ID. NO. 3	2×10^{-8} M
SEQ. ID. NO. 4	2×10^{-8} M

All patents and publications mentioned in this specification are indicative of the levels of those skilled in the art to which the invention pertains. All patents and publications are herein incorporated by reference to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference.

One skilled in the art will readily appreciate that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned as well as those inherent therein. The oligonucleotides, compounds, methods, procedures and techniques described herein are presently representative of preferred embodiments, are intended to be exemplary and are not intended as limitations on the scope. Changes therein and other uses will occur to those skilled in the art which are encompassed within the spirit of the invention or defined by the scope of the appended claims.

EXAMPLE 2

In Vivo Assay

The cellular assay for ERB-B2/*neu* described by Hudson, Ertl and Gill, Journal of Biological Chemistry 265:4389-4393 (1990) was used. The control region of the ERB-B2 gene was linked to the luciferase tester gene. After introduction by transfection, the resulting gene chimera was expressed in HeLa cells and was under ordinary control of the ERB-B2/*neu* promoter.

The example described was performed on an ERB-B2/*neu* luciferase chimera provided by G. N. Gill. The cellular experiments described in the reference were repeated in HeLa cells with the addition of NEU-specific TFOs and controls. NEU-specific TFOs and controls were added to the cell culture medium at 5 micromolar concentrations. In this analysis, the 3' propylamine derivatives were used to inhibit cellular nuclease activity. 10,000 cells were incubated for four hours with the ERB-B2/*neu* specific TFO or with a scrambled sequence isomer as a control. The gene activity was then monitored by measuring the amount of luciferase protein which remained in the cells after the four hour incubation period.

The results of the experiments are shown in the following table:

TFO	Concentration	Relative Luciferase Activity/10,000 Cells
Mock Treatment (saline treated cells)	0 uM	1.0
NEU41 (seq. ID 3)	5 uM	0.55 +/- .15
NEU28 (seq. ID 4)	5 uM	0.32 +/- 0.2
NEUctr (scrambled isomer of NEU28)	5 uM	0.95 +/- .15
Control 1 (scrambled Isomer of NEU41)	5 uM	1.05 +/-.25
Control 2 (scrambled another isomer of NEU41)	5 uM	1.1 +/- .2

Under the conditions of this assay the randomized isomer NEUctr has no effect on ERB-B2/*neu*. On the other hand, the TFOs NEU28 (seq. Id. No. 4) and NEU41 (seq. Id. No. 3) showed significant inhibition. NEU41 induced a 45% inhibition and NEU28 induced 68% inhibition of ERB-B2 promoter function as assessed by the cell system of Hudson et al. The randomized (scrambled) isomers (NEUctr, Control 1 and Control 2) do not bind to the target sequence in the NEU-B2 promoter region and do not show any binding or any inhibition activity of ERB-B2 promoter activity.

The target sequence for the TFOs of the present invention are shown in seq. Id. No. 1. In addition to the TFO sequences, SEQ. ID. Nos. 3 and 4, the NEUctr sequence and Control 1 and Control 2 are shown below.

NEUctr

3'A-TGTGTGGGTGTGGTGTGGGTGGTGGTGG-5'

CONTROL 1

3'A-TTGTGGTGGTGGTGTGGTGGTGGGGTTGGGTGGTGG-5'

CONTROL 2

3'A-TGGGTGGGGTGGGGTGGGGGGGGTGTGGGGTGTGGGGTG-5'

This example clearly shows that the TFOs are capable of: (i) entering the cell and the nucleus; (2) of binding duplex DNA targets; and (3) of inhibiting the function of the target. Thus, the claimed TFOs are

capable of *in vivo* inhibition of the promoter region of the ERB-B2/*neu* gene.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Hogan, Michael E.

(ii) TITLE OF INVENTION: Triplex Forming Oligonucleotide Reagents Targeted to the Neu Oncogene Promoter and Method of Use

(iii) NUMBER OF SEQUENCES: 10

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Fulbright & Jaworski

(B) STREET: 1301 McKinney, Suite 5100

(C) CITY: Houston

(D) STATE: Texas

(E) COUNTRY: U.S.A.

(F) ZIP: 77010-3095

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk

(B) COMPUTER: IBM PC compatible

(C) OPERATING SYSTEM: PC-DOS/MS-DOS

(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:

(B) FILING DATE:

(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Paul, Thomas D.

(B) REGISTRATION NUMBER: 32,714

(C) REFERENCE/DOCKET NUMBER: D-5393

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: 713/651-5151

(B) TELEFAX: 713/651-5246

(C) TELEX: 762829

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 74 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GCTTGCTCCC AATCACAGGA GAAGGAGGAG GTGGAGGAGG AGGGCTGCTT GAGGAAGTAT 60

AAGAATGAAG TTGT 74

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 54 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Triplex forming oligonucleotide

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

TGGTGTTGGT GGTGGTGGTG GTGGTGGGGT GGTTGTGGTT GTTTTTGTTT GTTG 54

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 41 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Triplex forming oligonucleotide

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TGGTGTTGGT GGTGGTGGTG GTGGTGGGGT GGTTGTGGTT G 41

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 28 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Triplex forming oligonucleotide

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

TGGTGTTGGT GGTGGTGGTG GTGGTGGG 28

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 54 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Triplex forming oligonucleotide

(iii) HYPOTHETICAL: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GTTGTTTGTT TTTGTTGGTG TTGGTGGGGT GGTGGTGGTG GTGGTGGTTG TGGT 54

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 41 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Triplex forming oligonucleotide

(iii) HYPOTHETICAL: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GTTGGTGTTG GTGGGGTGGT GGTGGTGGTG GTGGTTGTGG T 41

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 28 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Triplex forming oligonucleotide

(iii) HYPOTHETICAL: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GGGTGGTGGT GGTGGTGGTG GTTGTGGT 28

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 28 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Triplex forming oligonucleotide

(iii) HYPOTHETICAL: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GGTGGTGGTG GGTGTGGTGT GGGTGTGT 28

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 36 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Triplex forming oligonucleotide

(iii) HYPOTHETICAL: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GGTGGTGGGT TGGGGTGGTG GTGTGGTGGT GGTGTT 36

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 38 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Triplex forming oligonucleotide

(iii) HYPOTHETICAL: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GTGGGGTGTG GGGTGTGGGG GGGTGGGGTG GGGTGGGT 38

CLAIMS

What I claim is:

1. A method for inhibiting the proliferation of cells containing an erb B2/*neu* gene site, comprising the step of administering a therapeutic dose of triplex forming oligonucleotide (TFO), said TFO capable of binding to the major groove of a DNA duplex to form a colinear triplex with the promoter region of said erb B2/*neu* gene.

2. The method of claim 1, wherein the TFO forms the colinear triplex by binding to the section of the promoter region selected from the group consisting of a CAT box transcription factor binding site, a TATA box binding domain, a linker domain connecting the CAT box and TATA box and any combination thereof.

3. The method of claim 1, wherein the duplex DNA in the promoter region includes SEQ. ID. NO. 1 and its complementary strand.

4. The method of claim 1, wherein the TFO is SEQ. ID. NO. 2.

5. The method of claim 4, wherein the TFO is capped at the 3′ terminus with a modifier selected from the group consisting of polyamine, poly-L-lysine and cholesterol.

6. The method of claim 1, wherein the TFO is SEQ. ID. NO. 3.

7. The method of claim 6, wherein the TFO is capped at the 3′ terminus with a modifier selected from the group consisting of polyamine, poly-L-lysine and cholesterol.

8. The method of claim 1, wherein the TFO is SEQ. ID. NO. 4.

9. The method of claim 8, wherein the TFO is capped at the 3′ terminus with a modifier selected from the group consisting of polyamine, poly-L-lysine and cholesterol.

10. The method of claim 1, wherein the TFO is SEQ. ID. NO. 5.

11. The method of claim 10, wherein the TFO is capped at the 3′ terminus with a modifier selected from the group consisting of polyamine, poly-L-lysine and cholesterol.

12. The method of claim 1, wherein the TFO is SEQ. ID. NO. 6.

13. The method of claim 12, wherein the TFO is capped at the 3′ terminus with a modifier selected from the group consisting of polyamine, poly-L-lysine and cholesterol.

14. The method of claim 7, wherein the TFO is SEQ. ID. NO. 6.

15. The method of claim 14, wherein the TFO is capped at the 3′ terminus with a modifier selected from the group consisting of polyamine, poly-L-lysine and cholesterol.

16. The method of claim 1, wherein the cells are malignant cells.

17. The method of claim 1, wherein the cells are associated with diseases selected from the group consisting of neuroblastoma, glioblastoma and breast adenocarcinoma.

18. The method of claim 1, where the cells are non-transformed cells and are associated with conditions selected from the group consisting of scar tissue and psoriasis.

19. An oligonucleotide of the structure of SEQ. ID. NO. 2.

20. The oligonucleotide of claim 19 further comprising a cap at the 3′ terminus wherein said cap is selected from the group consisting of polyamine, poly-L-lysine and cholesterol.

21. The oligonucleotide of claim 20, wherein the cap is propylamine.

22. An oligonucleotide of the structure of SEQ. ID. NO. 3.

23. The oligonucleotide of claim 22 further comprising a cap at the 3′ terminus wherein said cap is selected from the group consisting of polyamine, poly-L-lysine and cholesterol.

24. The oligonucleotide of claim 23, wherein the cap is propylamine.

25. An oligonucleotide of the structure of SEQ. ID. NO. 4.

26. The oligonucleotide of claim 25 further comprising a cap at the 3′ terminus wherein said cap is selected from the group consisting of polyamine, poly-L-lysine and cholesterol.

27. The oligonucleotide of claim 26, wherein the cap is propylamine.

28. An oligonucleotide of the structure of SEQ. ID. NO. 5.

29. The oligonucleotide of claim 28 further comprising a cap at the 3′ terminus wherein said cap is selected from the group consisting of polyamine, poly-L-lysine and cholesterol.

30. The oligonucleotide of claim 29, wherein the cap is propylamine.

31. An oligonucleotide of the structure of SEQ. ID. NO. 6.

32. The oligonucleotide of claim 31 further comprising a cap at the 3′ terminus wherein said cap is selected from the group consisting of polyamine, poly-L-lysine and cholesterol.

33. The oligonucleotide of claim 32, wherein the cap is propylamine.

34. An oligonucleotide of the structure of SEQ. ID. NO. 7.

35. The oligonucleotide of claim 34 further comprising a cap at the 3′ terminus wherein said cap is selected from the group consisting of polyamine, poly-L-lysine and cholesterol.

36. The oligonucleotide of claim 35, wherein the cap is propylamine.

37. An oligonucleotide capable of forming a triplex by binding to the linker domain connecting a CAT box and a TATA box in the promoter region of the erb B2/*neu* gene, said oligonucleotide of sufficient length to disrupt CAT protein-TFIId interaction.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/09202

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 31/70; C07H 15/12, 17/00
US CL :514/44; 536/27
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/44; 536/27

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Chemical Abstracts, APS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Science, Volume 241, issued 22 July 1988, M. Cooney et al, "Site-Specific Oligonucleotide Binding Represses Transcription of the Human c-myc Gene in Vitro", pages 456-459, see entire document, especially page 458, col. 3, parag 6.	1-37
Y	Molecular and Cellular Biology, Volume 7, No. 7, issued July 1987, M. Tal et al, "Human HER2 (neu) Promoter:Evidence for Multiple Mechanisms for Transcriptional Initiation", 2597-2601, especially figure 1.	1-37

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
29 January 1993

Date of mailing of the international search report
02 FEB 1993

Name and mailing address of the ISA/
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231
Facsimile No. NOT APPLICABLE

Authorized officer
DEBORAH CROUCH, PH.D.
Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)★

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/09202

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any addtional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention frist mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.